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PROPORTION OF CONDENSED TANNIN IN DIGESTIVE PART OF SHEEP GIVEN PROTEIN MEALS AND POLYETHYLENE GLYCOL (PEG) ON *LEUCAENA PALLIDA* LEAVES BASE DIET

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ABSTRAK

Sebuah kajian telah dilakukan untuk melihat potensi sumber protein untuk mengurangi pengaruh negative tannin dibandingkan dengan PEG, melalui evaluasi konsentrasi tannin bebas dan tannin terikat dalam saluran pencernaan ternak. Sebanyak 24 anak domba dilibatkan dan ditempatkan secara acak dalam faktorial 2X2 sebanyak 6 ulangan. Ternak diberikan makanan campuran daun pallida selama empat minggu melalui automatic feeders. Hasil penilitian menunjukkan bahwa tepung ikan kelihatannya mengikat tannin bebas dalam saluran pencernaan melalui pembentukan ikatan protein- atau serat-tannin yang lebih tinggi dibandingkan dengan tepung bulu. PEG secara konsisten mengikat tannin bebas melalui peningkatan proporsi tannin yang terikat dalam bentuk ikatan serat-tannin. Olehnya itu, tepung ikan dan PEG berpotensu untuk menekan pengaruh negatif tannin.

Kata kunci : Protein suplemen, tannin, terikat serat atau protein

ABSTRACT

A study was conducted to evaluate the ability of protein meals to ameliorate the deleterious effects of tannin as compared to PEG through invesigation proportion of free tannin and bound tannin in the digestive tract of lambs. Twenty four wether lambs were involved and were randomly allocated in a factorial design of 2X2 within 6 replicates. They were fed with experimental diets for four weeks via continuos feeders. The results indicated that fish meal to some extent seems to bind tannins in the digestive tract of experimental animals by forming more protein-bound or fibre-bound tannin than feather meal did. PEG consistently bound free tannin in the digestive tract by enhancing the proportion of fibre-bound tannin. Therefore, fish meal and PEG are potentially reduce the the deleterious effects of tannins.

Key words : Protein meals, tannin, fibre- or protein-bound

I. INTRODUCTION

The positive effects of naturally bound-protein by tannin is attributed to the release of extra proteins from protein-tannins complex that have been dissociated post-ruminally. The extent to which protected proteins are released in the abomasum and small intestine is however still subject to some debate. It is likely that protein is not wholly or partially absorbed due to overprotection of the protein or reactivation of tannin in the small intestine (McNeill *et al*., 1998). Activated tannin binds not only exogenous protein but also endogenous protein (enzyme) and cause a morphological change and integrity in the intestine, particularly in jejunum and ileum (Dawson *et al*., 1999; Mbatha *et al*., 2002), resulting in absorption of nutrients. Some animals have ability to cope with dietary tannin by excreting proline rich proteins (PRP's) in their saliva as a natural defense mechanism (Mehansho *et al*. 1987). Proline forms an imide instead of amide bond, which cannot be hydrolysed by endogenous enzymes in mammals (Reed and Hutchens, 1994). Thereby, alternative proteins that mimic PRP because of similarities in their amino acids profiles might have ability to ameliorate the deleterious effect of tannins.

The objective of this study was to evaluate the ability of fish meal and feather meal as protein sources to possibly reduce the negative effects of tannins (pallida CT) in sheep through their ability to possibly bind free tannin in the digestive tract compared to PEG.

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II. MATERIALS AND METHODS

2.1. Preparation of Pallida Leaves

Pallida green leaves were harvested from a cultivated pastute in Spring-Summer season. Pallida trees were cut off about 1 meter above the ground level and then air dried in an air flow ventilated bunker to achieve about 90% of DM. The dry materials were then hand stripped to separate leaves from thick stem which were discarded. Edible leaf (fine stem and leaf) were further coarsely sieved to separate the leaf material from fine stem and was then stored in a sealed drums until fed to experimental animals.

2.2. Animals and Housing

Twenty four weather lambs of Border Leicester/Merino/Dorset cross (27.9±2.27 kg), about 5 months old, were used as experimental animals. The animals were housed in individual metabolism crates equipped with individual drinkers and feeders. They received pangola grass (*Digitaria decumbens* cv.) hay for about 3 days prior to the experimental period during which time they were fed the experimental diets for 4 weeks via continuous feeders.

2.3. Experimental Design and Diets

The twenty four animals were randomly allocated to one of four treatments groups in a factorial design (2x2) each with 6 replicates. The first factor was two different protein sources and the second factor was either with or without polyethylene glycol (PEG). Protein and PEG supplements provided 150 g and 75g/kg for crude protein (CP) DM of pallida leaves, respectively. Metabolically energy (ME) level of the protein supplements was equalized by using glucose. Therefore, such amount of protein supplements contributed about 3.2 MJ of ME. Basal diet (Pallida leaves) was mixed thoroughly with the supplements. The diet composition is presented in Table 1.

Table 1. Composition of The Experimental Diets As a Feed Fed to The Sheep

Ingredients	Fish meal + PEG	Fish meal - PEG	Feather meal+PEG	Feather meal-PEG
Pallida leaf (g)	1020.0	1020.0	1020.0	1020.0
Protein supplements (g):	263.6	263.6	0	0
Fish meal	0	0	177.7	177.7
Feather meal	0	0	76.5	76.5
Glucose	75	0	75	0
PEG (g)				
Chemical composition of	150.0	150.0	150.0	150.0
supplements:	3.2	3.2	3.2	3.2
Crude protein (g)				
ME (MJ)				

2.4. Measurement and Sampling Procedures

2.4.1 Intake and Liveweight

A 3-day adaptation period was allowed before the experiment began and ten days were allowed for adaptation to the experimental diets. Feed intakes were recorded daily from day 11 to day 17 of feeding period. Feed offered and feed refusals were recorded and sampled each day and later were analysed for DM and other chemical components. Initial liveweight of animals were recorded using an electronic scale before they were allocated to the treatment groups. The lambs were then weighed weekly during the 4-week feeding period just before the morning feeding.

2.4.2 Ruminal Fluid and Digesta Samples

Rumen fluid was collected by emptying the rumen of each animal individually after slaughter. The rumen contents were squeezed into the plastic container covered with nylon mesh to exclude the larger feed particles. pH of rumen fluid was immediately measured. Digesta samples were immediately taken from abomasum, duodenum, jejunum, ileum, caecum and rectum when the animals were terminated. Digesta from digestive tract was well mixed and sub-sampled and half time 50 mL jar. All digesta samples were immediately measured for pH and were frozen.

2.4.3 Chemical and Statistical Analysis

All bulked faecal samples were freeze dried for each lamb. Feed and faeces were samples were analysed for dry matter (DM), ash and organic matter (OM) contents. DM was estimated as the residue remaining after samples were dried at 65^{o}C for 48 h. Ash and OM were determined by incineration of samples in a muffle furnace at 550^{o}C for about 5 h, the loss in weight represented the OM content. Total nitrogen (N) contents of feed, faeces and urine samples were determined using a LECO auto analyzer (Leco Corporation, USA).

Analysis of tannin content of the diets and digesta samples was carried out using the Butanol/HCl method by Dalzell and Kerven (1998), in which pure CT of pallida was used as a standard. Tannin was categorized into free, protein bound, fibre bound and total tannin (Perez-Maldonado, 1994). More detail of the analysis method is presented in Rusdi (2004).

The data were analyzed by analyses of variance as a factorial design (Steel and Torrie, 1980) by means of General Linear Model of SAS packages (SAS, 1998). The LSD was used to compare means between treatments.

III. RESULTS AND DISCUSSION

3.1. Chemical Composition of Feeds

Chemical composition of pallida leaves and diets for animals is presented in Table 2. The CT content of pallida was higher than actual diet fed to all animals.

Table 2. Chemical Composition (g/kg DM) of Pallida Leaves and Mixture Diets Fed to Animals

Criteria	Pallida leaf	Fish meal		Feather meal	
		+PEG	-PEG	+PEG	-PEG
Dry matter	937	942	939	941	938
Organic matter	881	855	865	851	865
Nitrogen	40	48	50	48	51
Condensed tannin					
Free	129	74	112	72	107
Protein bound	11	16	12	12	10
Fibre bound	10	8	4	10	7
Total	150	98	128	94	124

3.2. pH Values of Digesta of Digestive Tract

Protein sources did not affect the pH values of rumen fluid, digesta from duodenum, jejunum or ileum and the urea level in blood ($P>0.05$; Table 3). However, fish meal significantly ($P<0.05$) increased pH in the digesta of abomasum and caecum ($P<0.01$). Inclusion of PEG in the diets did not influence the pH values of digesta from digestive tract ($P>0.05$), but decreased those of rumen fluid ($P<0.05$). PEG consistently increased the rumen-ammonia concentration. There was no interaction between protein and PEG on these variables ($P>0.05$).

3.3. The Concentration of CT along Digestive Tract

The concentration of CT in three different fractions (free, fibre and protein bound) and total tannin is presented in Table 4. Protein sources had no significant effect on the concentration of CT along digestive tract ($P>0.05$). PEG treatment, on the other hand, resulted in a significant effect on the concentration of CT in the fibre bound form, particularly in digesta samples were taking from duodenum, terminal ileum and rectum ($P<0.01$). PEG significantly elevated the concentration of CT in the form fibre bound in duodenum and ileum ($P<0.05$) and in the rectum ($P<0.01$). The concentration of free tannin fraction decreased as digesta move down to the lower digestive tract and it disappeared when the digesta reached to the terminal ileum and the rectum (faeces). While other fractions behaved the opposite with free tannin concentration.

An *in vitro* study of tannin complexing agents by Makkar *et al*. (1995) found that the most effective agent was PEG followed by polyvinyl pyrrolidone (PVP) and polyvinyl polypyrrolidone (PVPP). The PEG has been used in many studies to bind and inactivate tannin in plant material fed to animals (McNeill *et al*., 1998; Landau *et al*., 2000; Gibious, 2001). Calculated ratio of PEG and tannin in this study was based on the previous study using the pallida leaves by McNeill *et al*. (1998) and Gobius (2001). They concluded that 75g of PEG/kg DM of pallida leaves (15% of CT) was effectively inactivated CT which allow sheep to perform better than intact tannin. While the *in vitro* result in Rusdi (2004) showed that 0.5 mg of PEG was effectively bind 1 mg of CT from pallida. The calculated ratio of PEG to tannin in the present study was 1:2 on the total tannin basis (Table 1) , which was comparable to 1:2 for sheep (Silanikove *et al*., 1994), 1:4 to 1:8 for goat (Silanikove *et al*.,1997) and 1:1.6 for heifers (Landau *et al*., 2000).

Table 3. The Values of pH in Different Part of Digestive Tract of Animals Fed Mixture of Pallida, Either Fish Meal or Feather Meal and with or Without Peg

Parameters	Fish meal		Feather meal		SEM[#]	Significance [@]		
	+PEG	-PEG	+PEG	-PEG		S	P	SXP
pH values :								
- Rumen	6.5	6.8	6.4	6.8	0.06	NS	**	NS
- Abomasum	3.6	3.3	2.8	3.1	0.15	**	NS	NS
- Duodenum	4.3	3.8	3.3	3.9	0.32	NS	NS	NS
- Jejunum	6.9	6.5	6.2	6.6	0.48	NS	NS	NS
- Ileum	8.0	8.0	8.0	7.9	0.05	NS	NS	NS
- Caecum	7.5	7.5	7.2	7.3	0.06	**	NS	NS

[@] significance of difference between mean :

* $P<0.05$, ** $P<0.01$, NS not significant, S protein source, P PEG and SXP interaction of S and P; [#] Standard error of mean

There was no significant effect of treatments on the concentration of CT (free, fibre and protein bound) in the abomasal and jejunal digesta samples (P>0.05; Table 4), but there was a tendency of enhancement of protein bound due to the inclusion of fish meal in the diet. In contrast, supplementation with PEG had a significant effect (P<0.05) on the concentration of CT in the fibre bound form from duodenal, ileal and rectal digesta samples. PEG resulted in an increased concentration of fibre bound tannin form in the duodenum, ileum and rectum (P<0.05), as CT moved down into the digestive tract toward the ileum. PEG also increased fibre fraction in the rectum but the values were lower than that the terminal ileum. This evidences support the fact as found in many reports that PEG does effectively bind tannin in the digestive tract and the effectiveness of PEG mainly depends on the tannin-PEG ratio exist in the digestive tract.

The greatest proportion of free tannin was recorded in the abomasum. This was related to the acidic condition of abomasum (Table 3), causing an extensive dissociation of tannin complexes and released more tannin in the free form. This follows the general belief of dissociation characteristics of tannin complexes, which occurs in the acidic environment of abomasum (Mangan, 1988). Interestingly, the amount of free tannin decreased as the digesta moved down to the lower part of digestive tract and a concomitant with an increased fraction of fibre and protein bound form (Table 4). Free tannins were not detected in digesta from terminal ileum and rectum. This trend is an agreement with the previous study in sheep by Ahn (1990) or Perez-Maldonado (1994). It is postulated that the disappearance of free tannins has partly been bound with PEG or protein meal since the tannin bond fibre or protein increased in the present results similar to that reported by Wang *et al*. (1996) who found that protein-tannin complexes were still found in the terminal ileum. It also is possible that free tannin or the others fraction of tannin that have been degraded by intestinal microflora and changed to the chemical form that absorbable in the digestive tract (Perez-Maldonado, 1994), and were voided in the urine via liver and kidney (Butler *et al*., 1984). It is unlikely that the absence of free tannins in the digestive tract was due to its binding to carbohydrates unextractable either by acetone or sodium dodecyl sulphate as reported by

Table 4. Tannins Concentration (% DM) of Digesta Samples from Different Part of Digestive Tract of Animals Feed Mixture of Pallida, either Fish Meal or Feather Meal and With or Without PEG

Parameters	Fish meal		Feather meal			Significance[@]		
	+PEG	-PEG	+PEG	-PEG	SEM[#]	S	P	SXP
Abomasum								
Free	8.9	11.2	11.2	10.0	0.89	NS	NS	NS
Fibre bound	0.6	0.6	0.5	0.5	0.06	NS	NS	NS
Protein bound	6.3	7.2	6.9	5.7	0.68	NS	NS	NS
Total	15.8	19.0	18.6	16.3	1.37	NS	NS	NS
Duodenum								
Free	5.4	5.2	5.5	5.5	0.95	NS	NS	NS
Fibre bound	0.9	0.6	0.7	0.5	0.01	NS	*	NS
Protein bound	4.8	7.2	5.9	7.5	0.67	NS	**	NS
Total	11.0	13.0	12.0	13.4	1.01	NS	NS	NS
Jejunum								
Free	0.7	1.9	1.8	2.2	0.59	NS	NS	NS
Fibre bound	1.5	1.0	1.6	1.3	0.21	NS	NS	NS
Protein bound	6.6	7.1	6.6	5.7	0.61	NS	NS	NS
Total	8.8	9.9	9.9	9.2	0.83	NS	NS	NS
Ileum								
Free	ND	ND	ND	ND	ND	ND	ND	ND
Fibre bound	2.1	1.6	2.1	1.3	0.28	NS	*	NS
Protein bound	6.8	7.5	7.9	8.3	0.60	NS	NS	NS
Total	8.9	9.0	10.1	9.6	0.47	NS	NS	NS
Rectum (Faeces)								
Free	ND	ND	ND	ND	ND	ND	ND	ND
Fibre bound	2.0	1.3	2.0	1.1	0.14	NS	**	NS
Protein	3.3	4.0	3.6	3.4	0.16	NS	NS	*
Total	5.3	5.3	5.6	4.5	0.34	NS	*	NS

[@] Significance of difference between mean :
* P<0.05, ** P<0.01, NS not significant, ND not detected, S protein source, P PEG and SXP interaction of S and P, [#] Standard error of mean

Ahn (1990), due to other fractions of tannins have been quantified in the form of fibre bound and protein bound in the present study by using acetone and SDS reagent respectively. Distel and Provenza (1991) had difficulties to analyse tannin in the faeces of goat and found only 6% of ingested CT was recovered even using a powerful solution like acetic acid, SDS and acetone or ether. Then they concluded that the main problem of working with such samples is the extraction of tannin *per se*. Perez-Maldonado (1994) suggested that some of label tannin did not recover in the faeces or urine because they were completely or partially not reactive with Butanol-HCL reagent or because the absorbed free tannins were extensively degraded by the liver before being excreted in the urine. Therefore, the abrosption or degradation rate of the investigated tannin was about 70-80% occurred during the passage to the faeces (Perez-Maldonado, 1994).

Other studies suggested that CT from sorghum, leucaena and mulga have a marked loss in the gut of sheep which was about 89, 92 and 88% for sorghum, leucaena and mulga respectively and 70-80% of the losses mainly occurred in post ruminal tract (Goodchild, 1989). Ahn (1990) recorded about 84.6 and 85.5% CT losses in sheep given dried and frozen calliandra respectively. In a study using quebracho tannin by Robbins *et al*. (1991), it was recorded that only 70 to 77% of high molecular fraction was recovered in domestic sheep. Condensed tannin, in many cases, was at greater extent degraded and absorbed in the digestive tract, particularly for a low molecular weight (Foley *et al*., 1999), which varied mainly depending on the species of consumers and type of tannin and diets. Such evidence clearly confirmed the general hypothesis that CT exerted their deleterious effect in the small intestine where nutrients absorption process is taking place, which could be more severe since free tannin presents in a significant amount on this part of digestive tract and therefore efforts to permanently bind such tannins through digestive tract give a significant benefit in animal nutrition and need to be explored to achieve an appropriate feeding system.

IV. CONCLUSION

Inclusion of protein source in tannin-containing diet, to some extent bind free-tannin in the digestive tract to which fish meal seemed to bind more tannin than feather meal did. PEG consistently bind free-tannin in digestive tract by enhancing the proportion of fibre-bound tannin. Thus, additional protein and/or PEG is clearly to reduce the inhibitory effect of pallida tannins in the digestive tract.

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